PRODUCTION OF AMIDE COMPOUND BY USING MICROORGANISM

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ABSTRACT

PROBLEM TO BE SOLVED: To provide a method for producing an amide corresponding to a nitrile compound from the nitrile compound by utilizing cell bodies of microorganisms producing a nitrile hydratase, or a treated material of the cell bodies, by which a reaction liquid having a higher amide concentration is obtained by using smaller amount of the cell bodies.

SOLUTION: This method for producing acrylamide from acrylonitrile by utilizing cell bodies of microorganisms producing a nitrile hydratase, or a treated material of the cell bodies comprises addition of the acrylonitrile to a reaction liquid so that the concentration of the acrylonitrile may be not less than the solubility of the acrylonitrile at the time of the start or on the way of the reaction for forming the acrylamide by bringing the cell bodies of the microorganisms capable of keeping the nitrile hydratase activities after treating the microorganisms in 30 wt.% acrylamide aqueous solution at 10°C for 60 min, or the treated material thereof, into contact with the acrylonitrile in an aqueous medium.

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PROBLEM TO BE SOLVED: To provide a method for producing an amide corresponding to a nitrile compound from the nitrile compound by utilizing cell bodies of microorganisms producing a nitrile hydratase, or a treated material of the cell bodies, by which a reaction liquid having a higher amide concentration is obtained by using smaller amount of the cell bodies.

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CLAIMS

[Claim(s)]
[Claim 1] It is the approach of manufacturing acrylamide from acrylonitrile using the fungus body or its fungus body processing object of the microorganism which produces nitril hydratase. In 30% of the weight of an acrylamide water solution, the fungus body of this microorganism At 10 degrees C Also after processing for ** 60 minutes, the activity of nitril hydratase A fungus body or a fungus body processing object of this microorganism which is held So that the acrylonitrile concentration in the middle of the time of reaction initiation of the reaction which makes acrylonitrile contact in an aquosity medium and makes acrylamide generate, or a reaction may turn into more than the saturated concentration of the acrylonitrile in the inside of an aquosity medium The manufacture approach of the acrylamide characterized by adding acrylonitrile to reaction mixture.
[Claim 2] The manufacture approach according to claim 1 characterized by adding acrylonitrile so that the acrylamide concentration in the reaction mixture after reaction termination may become 30 % of the weight or more.
[Claim 3] It is the approach of manufacturing methacrylamide from methacrylic nitril using the fungus body or its fungus body processing object of the microorganism which produces nitril hydratase. In 30% of the weight of an acrylamide water solution, the fungus body of this microorganism At the time of reaction initiation of the reaction which contacts a fungus body or a fungus body processing object of this microorganism which holds the activity of nitril hydratase also after processing for 60 minutes at 10 degrees C to methacrylic nitril in an aquosity medium, and makes methacrylamide generate Or the manufacture approach of the methacrylamide characterized by adding methacrylic nitril to reaction mixture so that the methacrylic nitril concentration in the middle of a reaction may turn into more than the saturated concentration of the acrylonitrile in the inside of an aquosity medium.
[Claim 4] It is the approach of manufacturing a croton amide from croton nitril using the fungus body or its fungus body processing object of the microorganism which produces nitril hydratase. In 30% of the weight of an acrylamide water solution, the fungus body of this microorganism At 10 degrees C Also after processing for ** 60 minutes, the activity of nitril hydratase A fungus body or a fungus body processing object of this microorganism which is held So that the croton nitril concentration in the middle of the time of reaction initiation of the reaction which makes croton nitril contact in an aquosity medium, and makes a croton amide generate, or a reaction may turn into more than the saturated concentration of the croton nitril in the inside of an aquosity medium The manufacture approach of the croton amide characterized by adding croton nitril to reaction mixture.
[Claim 5] From claim 1 to which nitril hydratase which this microorganism that holds the activity of nitril hydratase has discovered also after processing the fungus body of the microorganism which produces nitril hydratase for 60 minutes at 10 degrees C in 30% of the weight of an acrylamide water solution is characterized by having the amino acid sequence of array number 1 publication of an array table, or the amino acid sequence of array number 2 publication of an array table in alpha subunit to the manufacture approach according to claim 4
[Claim 6] This microorganism that holds the activity of nitril hydratase also after processing the fungus body of the microorganism which produces nitril hydratase for 60 minutes at 10 degrees C in 30% of the weight of an acrylamide water solution A Nocardia (Nocardia) group, the Corynebacterium (Corynebacterium) group, A bacillus (Bacillus) group, Bacillus of thermophilic nature, the Pseudomonas (Pseudomonas) group, A micrococcus (Micrococcus) group, a RODOKO@KKASU (Rhodococcus) group, The Acinetobacter (Acinetobacter) group, the Xanthobacter (Xanthobacter) group, A streptomyces (Streptomyces) group, a rhizobium (Rhizobium) group, A klebsiella (Klebsiella) group, the Enterobacter (Enterobacter) group, The Erwinia (Erwinia) group, the Aeromonas (Aeromonas) group, The

Citrobacter (Citrobacter) group, an achromobacter (Achromobacter) group, From claim 1 characterized by being a microorganism belonging to the Agrobacterium (Agrobacterium) group or the Pseudonocardia (Pseudonocardia) group to the manufacture approach according to claim 4
[Claim 7] From claim 1 characterized by this microorganism that holds the activity of nitril hydratase also after processing the fungus body of the microorganism which produces nitril hydratase for 60 minutes at 10 degrees C in 30% of the weight of an acrylamide water solution being a gene recombination microorganism which made the nitril hydratase gene of the Pseudonocardia thermostat filler (Pseudonocardia thermophila JCM3095) origin discover to the manufacture approach according to claim 4

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DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the reaction approach of making the amide compound which corresponds from a nitryl compound in an aquosity medium generating, using the microorganism fungus body which has nitril hydratase activity, or its fungus body processing object. It is related with the approach of manufacturing the amide compound which corresponds to a detail from a nitryl compound efficiently more by setting up the concentration of this nitryl compound in the middle of the time of reaction initiation, or a reaction more than the saturated concentration of this nitryl compound in the inside of an aquosity medium.
[0002]
[Description of the Prior Art] The nitril hydratase which is the enzyme which has the nitril hydration activity which hydrates a nitrile group in recent years and is changed into an amide group is discovered. The method of manufacturing the amide compound which corresponds from a nitryl compound in an aquosity medium using a fungus body or its fungus body processing object of the microorganism containing this enzyme and this enzyme etc. is learned (JP,62-21519,B). By the manufacture approach of the amide compound using nitril hydratase, there is a merit with high invert ratio and selectivity of a nitryl compound compared with the chemical approach till then. However, the amide compound which is the nitryl compound and product which serve as a substrate of an enzyme reaction on the other hand is an organic compound which generally has strong living thing toxicity, and carrying out deactivation of this enzyme quickly is conventionally pointed out as the concentration in the system of reaction becomes high. Therefore, it faced manufacturing an amide compound from a nitryl compound industrially, and it has been thought important by keeping low the concentration of the nitryl compound in the system of reaction, and an amide compound to hold nitril hydratase activity to stability and to advance a reaction smoothly.
[0003] If an example is given, the report (heredity, a separate volume No. 1, 36 pages to 45 pages, March, 1988) of Nagasawa and Yamada is known as an approach of manufacturing an amide compound industrially from a nitryl compound. The approach of manufacturing acrylamide from acrylonitrile in this report (from 38 pages, the right column, and the bottom to the 8th line) "a resting-bacterium object is made to react with acrylonitrile in 5-15 degrees C and the pH7.0 neighborhood with the concentration of 20mg/ml. It is indicated that it carries out division addition with time with advance of a reaction at this time since high-concentration acrylonitrile checks nitril hydratase activity."
[0004] Although the method of making this hydration reaction that makes acrylonitrile or a methacrylonitrile a substrate perform in JP,56-38118,B under the low temperature from the freezing point to 15 degrees C is indicated, the substrate concentration in that case is indicated it is "appropriate for a substrate" in this enzyme reaction to add so that it may set in the system of reaction and may always be in a dissolution condition. Furthermore, "what is necessary is just to drop acrylonitrile or a methacrylonitrile at the bottom of churning, maintaining temperature at the aqueous suspension containing said immobilized cell at freezing point -15 degree C, although a feeding method is usually taken when based on a batch process" is indicated, and the need for division addition of a nitryl compound is shown.
[0005] By JP,57-1234,B, from acrylonitrile or a methacrylonitrile, it faces manufacturing acrylamide or methacrylamide continuously, the approach of supplying the concentration of a nitryl compound continuously in the amount within the limits dissolved to a reaction mixture is indicated, and the importance of controlling the concentration of a nitryl compound low also in the official report concerned is shown.
[0006] When carrying out hydration of the nitryl compound in an aquosity medium using nitril hydratase and

manufacturing an amide compound, accumulating the amide compound generated with advance of a reaction is continued into an aquosity medium. The maximum concentration of the amide compound to generate is determined by the property and reaction temperature of nitril hydratase of each microorganism origin. That is, when this enzyme and an amide compound are contacted in the reaction temperature, more than the lower limit of the amide compound concentration to which this enzyme deactivates immediately, the concentration of the amide compound in reaction mixture does not become high. In addition, the concentration of this amide compound will be described as the amide compound concentration of a limitation from now on.

[0007] It is in the report of above-mentioned Nagasawa and Yamada, saying, "Production of the acrylamide which reaches also to 40% was made impossible depending on the current chemical approach, and the effectiveness of the enzymatic process as a industrial process of acrylamide was suggested here strongly" (from 39 pages, the left column, and a top to the 2nd line). Thus, it is important industrially that the concentration of the amide compound at the time of reaction termination is high, and nitril hydratase with the high amide compound concentration of this limitation is useful.

[0008] When it is made to react by controlling the nitryl compound concentration in reaction mixture low, in order for the time amount to which nitril hydratase contacts a nitryl compound and an amide compound to become long relatively and to store up amide compound concentration to the amide compound concentration of a limitation, it is necessary to make [many] nitril hydratase activity in the system of reaction relatively. Since increase of the amount of the enzyme used becomes disadvantageous for manufacture of a industrial amide compound from a cost side, the reaction approach of controlling the nitryl compound concentration in reaction mixture low cannot necessarily be said to be the efficient reaction approach.

[0009] By the way, it is almost the case that the microorganism strain which has nitril hydratase activity has the amidase which is the enzyme which hydrates an amide compound to a corresponding carboxylic-acid compound. For this reason, when the amide compound which corresponds from a nitryl compound using this microorganism strain is made to generate, it is known that the carboxylic-acid compound which corresponds by this amidase sub**. In order to avoid this, the variation microorganism strain which does not have amidase activity needed to be acquired, and the preparation approach of a microorganism fungus body that amidase activity became low relatively needed to be devised.

[0010]

[Problem(s) to be Solved by the Invention] The technical problem of this invention is to offer an efficient approach rather than manufacturing the amide compound which corresponds from a nitryl compound using a microorganism.

[0011] The purpose of this invention is in the approach of manufacturing the amide which corresponds from a nitryl compound using the fungus body or its fungus body processing object of the microorganism which produces nitril hydratase to obtain the reaction mixture of higher amide concentration by smaller cell mass.

[0012]

[Means for Solving the Problem] this invention persons found out that the resistance over a corresponding nitryl compound also had [nitril hydratase above high to some extent] the high amide compound concentration of the above-mentioned limitation. And if the reaction for which the resistance over such an amide compound generates the amide which corresponds from a nitryl compound using the fungus body or its fungus body processing object of the microorganism which has nitril hydratase above high to some extent is performed In the reaction mixture, it became clear that the reaction rate of this enzyme increased relatively as deactivation of this enzyme by strong nitryl compounds of living thing toxicity, such as acrylonitrile and a methacrylonitrile, did not progress quickly but the concentration of this nitryl compound in an aquosity medium became high. That is, the reaction approach which adds acrylonitrile to reaction mixture so that it may become more than the saturated concentration of the nitryl compound in the inside of an aquosity medium without the need of always controlling low the concentration of the nitryl compound in the system of reaction currently performed conventionally was newly found out.

[0013] And as a result of examination by this invention persons, according to the newly found-out reaction approach, when the same amount of enzymes (active mass) was used, it was checked in that this nitryl compound is more convertible for a corresponding amide compound in a short time as compared with the conventional approach, and the more nearly little amount of enzymes (active mass) that the amide compound water solution of higher concentration can be obtained. this invention persons came to complete this invention based on the above knowledge.

[0014] Namely, this invention is the approach of manufacturing an amide compound from a nitryl compound using the

fungus body or its fungus body processing object of the microorganism which produces nitril hydratase. A fungus body or a fungus body processing object of this microorganism which holds the activity of nitril hydratase also after processing the fungus body of this microorganism for 60 minutes at 10 degrees C in 30% of the weight of an acrylamide water solution So that the nitryl compound concentration in the middle of the time of reaction initiation of the reaction which makes a nitryl compound contact in an aquosity medium, and makes an amide compound generate, or a reaction may turn into more than the saturated concentration of the nitryl compound in the inside of an aquosity medium Reaction mixture is provided with the manufacture approach of the amide compound characterized by adding a nitryl compound.

[0015] It faces manufacturing the amide compound which corresponds from a nitryl compound in an aquosity medium using the fungus body or fungus body processing object of a microorganism which produces nitril hydratase according to this invention. [in the middle of / its / the time of initiation of the reaction which generates the amide compound which corresponds from a nitryl compound] By adding this nitryl compound in the system of reaction more than the saturated concentration of this nitryl compound in the inside of an aquosity medium, it becomes efficiently possible to generate the amide compound which corresponds from a nitryl compound for a short time by high concentration.

[0016]

[The gestalt of invention implementation] Hereafter, it outlines about the detail of this invention. The carbon number of the nitryl compound in this invention is the nitryl compound of 2-8. Also in it, acrylonitrile, a methacrylonitrile, and croton nitril can be especially mentioned as a suitable example.

[0017] If the activity of this nitril hydratase is held also after processing the fungus body of the microorganism which has the capacity which generates the amide compound which hydrolyzes a nitryl compound and corresponds, and produces nitril hydratase in 30% of the weight of an acrylamide water solution for 60 minutes at 10 degrees C, especially a limit will not be carried out to the nitril hydratase in this invention. Preferably, the nitril hydratase which has the amino acid sequence of array number:1 publication of an array table or the amino acid sequence of array number:2 publication can be mentioned in the alpha subunit.

[0018] The resistance over the amide compound of the nitril HIDORATA ase of various microorganisms is various. While the nitril hydratase which deactivates completely is after 60 minutes in 10% of the weight of an acrylamide water solution when acrylamide is taken for an example as an amide compound, and processing nitril hydratase at 10 degrees C in an acrylamide water solution, there is nitril hydratase which does not deactivate even if it processes for 60 minutes in 50% of the weight of an acrylamide water solution. Thus, the acrylamide concentration of the limitation of nitril hydratase mentioned above changes with each nitril hydratases.

[0019] It is important industrially that the concentration of the amide compound at the time of reaction termination is high, and especially use of 30% of the weight or more of nitril hydratase has the effective acrylamide (activity is held) concentration which does not deactivate after processing for 60 minutes at 10 degrees C in an acrylamide water solution. Moreover, when producing an amide compound industrially, it is advantageous from a cost side to present a reaction with the fungus body or its fungus body processing object of the microorganism which produces nitril hydratase.

[0020] That is, in this invention, when the fungus body of the microorganism which produces nitril hydratase is processed for 60 minutes at 10 degrees C in an acrylamide water solution, it is desirable to use the fungus body or its fungus body processing object of the microorganism to which the upper limit of the acrylamide concentration by which the activity of nitril hydratase is held produces 30% of the weight or more of nitril hydratase.

[0021] Especially a limit will not be carried out, if the microorganism which holds the activity of nitril hydratase even if it processes for 60 minutes at 10 degrees C in 30% of the weight of the acrylamide water solution in this invention is a microorganism holding the activity of nitril hydratase even if it produces the nitril hydratase which has the capacity which generates the amide compound which hydrolyzes a nitryl compound and corresponds and processes the fungus body of this microorganism for 60 minutes at 10 degrees C in 30% of the weight of an acrylamide water solution. What is necessary is just the microorganism which is producing preferably the nitril hydratase which has the amino acid sequence of array number:1 publication of an array table, or the amino acid sequence of array number:2 publication in the alpha subunit. Specifically A Nocardia (Nocardia) group, the Corynebacterium (Corynebacterium) group, A bacillus (Bacillus) group, Bacillus of thermophilic nature, the Pseudomonas (Pseudomonas) group, A micrococcus (Micrococcus) group, the RODOKO@KKASU (Rhodococcus) group represented by the RODOKUROUSU (rhodochrous) kind, The Acinetobacter (Acinetobacter) group, the Xanthobacter (Xanthobacter) group, A streptomyces

(Streptomyces) group, a rhizobium (Rhizobium) group, A klebsiella (Klebsiella) group, the Enterobacter (Enterobacter) group, The Erwinia (Erwinia) group, the Aeromonas (Aeromonas) group, The Citrobacter (Citrobacter) group, an achromobacter (Achromobacter) group, The microorganism belonging to the Pseudonocardia (Pseudonocardia) group represented by the Agrobacterium (Agrobacterium) group or the thermostat filler (thermophila) kind can be mentioned as a suitable example.

[0022] Moreover, it is contained in the microorganism which produces the nitril hydratase as used in the field of [the transformant which made the nitril hydratase gene which carried out cloning from this microorganism discover by the host of arbitration] this invention. In addition, although Escherichia coli (Escherichia coli) is mentioned to the host of arbitration here as an example of representation like the below-mentioned example, other microorganism strain, such as the genus Bacillus, such as a Bacillus subtilis (Bacillus subtilis) instead of a limiting-to especially Escherichia coli thing, and yeast, an Actinomyces, is contained. As an example of such a thing, MT-10822 (the bacteria stock is deposited with National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology of 1-1-3, Higashi, Tsukuba-shi, Ibaraki-ken, the Ministry of International Trade and Industry, as trust number FERM BP-5785 on February 7, 1996 based on Budapest Treaty about international acknowledgement of deposition of the microorganism on patent procedure.) is mentioned. Moreover, it is contained in the microorganism which produces the nitril hydratase as used in the field of [the transformant which made the nitril hydratase of the variant which raised further amide compound resistance, nitryl compound resistance, and temperature resistance a permutation, deletion, and by deleting or inserting using recombinant DNA technology from 1 of the configuration amino acid of this enzyme, or the amino acid of others two or more pieces discover] this invention.

[0023] The manufacture approach of this invention is faced using the microorganism in this invention, and the fungus body or fungus body processing object of this microorganism is used. What is necessary is just to prepare a fungus body in the field of molecular biology, bionics, and gene engineering using a well-known general approach. For example, after carrying out inoculation of this microorganism to usual liquid media, such as LB culture medium and M9 culture medium, it is made to grow at suitable culture temperature (for 50 degrees C or more to be sufficient generally, in the case of thermophilic bacteria, although it is 20 degrees C - 50 degrees C.), then the method of dissociating and collecting these microorganisms and obtaining them from culture medium, according to centrifugal separation, is mentioned.

[0024] Moreover, the fungus body processing object of the microorganism in this invention points out the fixed object which carried out separation purification of the nitril hydratase activity fraction of the extract and the grinding object of the above-mentioned microorganism fungus body, this extract, or a grinding object, was obtained, and fixed the separation-after **** object, and the extract, the grinding object and the back separation object of this microorganism fungus body or this fungus body using suitable support, and these are equivalent to the fungus body processing object of this invention, as long as it has the activity of nitril hydratase.

[0025] A batch reaction can also be presented with the fungus body or fungus body processing object of a microorganism in this invention, and a successive reaction can also be presented with it. Moreover, you may use as a suspension floor and may use as the fixed bed. Especially a limit is not carried out unless the fungus body of this microorganism in the inside of the reaction mixture in that case or the concentration of a fungus body processing object causes trouble to mixing of an aquosity medium and a nitryl compound.

[0026] The aquosity medium in this invention shows the water solution in which the hydroxide, the amide compound, etc. of mineral salt and alkali metal, such as a buffer and sulfates, such as water or phosphate, and a carbonate, were dissolved by suitable concentration.

[0027] In this invention, it is important by being supplied from a nitril phase that long duration maintenance of the concentration is carried out relatively before and after saturated concentration at the same time the nitryl compound in an aquosity medium is consumed with advance of a reaction. for this reason, a batch reaction or a successive reaction -- it is desirable to add a nitryl compound serially so that the whole quantity of a required nitryl compound may be put in block at the time of reaction initiation, and it may add in any case or the nitryl compound concentration in the inside of the aquosity medium in the middle of a reaction may be maintained more than saturated concentration. Moreover, it is important to use suitable mixed equipments, such as a rotary wing and a line mixer, and to also make the aquosity medium phase divided into the bottom of standing at a two phase and a nitril phase fully mix.

[0028] The minimum of the concentration of the nitryl compound in the case of carrying out package addition of the nitryl compound at the time of reaction initiation should just be more than the saturated concentration of this nitryl

compound at the time of reaction initiation. What is necessary is on the other hand, not to limit especially the upper limit of the concentration and just to determine it as arbitration with the amide compound concentration and nitryl compound concentration at the time of the reaction termination to assume. Moreover, an unreacted nitryl compound is also removable from reaction mixture after a reaction with means, such as distillation. Therefore, even when the amide compound concentration at the time of the reaction termination to assume is reached, a nitryl compound can also be added so that a nitryl compound may become superfluous.

[0029] When acrylonitrile is a nitryl compound, since the saturated concentration of this compound to water is about 7 % of the weight, specifically, about 7 % of the weight or more and 50% of the weight or less of the range is suitable for it. Moreover, when a methacrylonitrile or croton nitril is a nitryl compound, since the saturated concentration of these compounds to water is about 2 % of the weight, about 2-% of the weight or more 50 or less % of the weight of the range is suitable for it.

[0030] Although the reaction in this invention is generally performed under ordinary pressure, in order to raise the solubility of the nitryl compound to the inside of an aquosity medium, it can also be performed under pressurization. Moreover, although it will not be restricted about reaction temperature especially if it is more than the freezing point of an aquosity medium, 50 degrees C is more preferably performed from 0 degree C within the limits of 10 to 30 degrees C. although pH is not restricted on the other hand especially as long as nitril hydratase activity is maintained -- desirable -- pH10 from pH6 -- it is within the limits of pH7 to pH9 more preferably.

[0031] In the reaction which generates the amide compound in the approach of this invention, a nitril hydration reaction advances good rather so that nitril hydratase may not deactivate immediately. Since it is supplied from a nitril phase at the same time the nitryl compound about it and in an aquosity medium phase is consumed with advance of a reaction, the concentration is always maintained near saturated concentration. For this reason, since a nitril hydration reaction advances at the maximum reaction rate in that reaction temperature, or the rate near it, a short time and the amide compound which corresponds from this nitryl compound efficiently will generate relatively.

[0032] Moreover, after adding a nitryl compound more than the saturated concentration in the inside of an aquosity medium, with advance of a reaction, the nitryl compound concentration in the system of reaction falls gradually, and turns into below the saturated concentration in an aquosity medium. Although a reaction rate also falls gradually by this, since a nitril hydration reaction advances at the maximum reaction rate or the rate near it, a short time and the amide compound which corresponds from this nitryl compound efficiently will generate substantially till the point in time.

[0033] Furthermore, according to the manufacture approach of this invention, the secondary effectiveness that generation of the corresponding carboxylic-acid compound by the amidase in a microorganism fungus body is controlled relatively can also be acquired by making high concentration of this nitryl compound in an aquosity medium.

[0034] After the obtained amide compound separates a fungus body or a fungus body processing object from reaction mixture by the usual well-known approaches, such as centrifugal separation, it may be used as a water solution as it is, may be condensed by approaches, such as membrane concentration and spray dry concentration, and may obtain a crystal. Moreover, after separating a fungus body or a fungus body processing object, the purity of an amide compound can also be further raised by using activated carbon, ion exchange resin, ion exchange membrane, etc., and removing impurities, such as coloring matter.

[0035]

[Example] Although the following examples explain this invention to a detail further, this invention is not limited at all by the following examples. In addition, the HPLC analysis in each example and the example of a comparison is Jasco Finepak as a column. SIL 10mM phosphoric-acid water solution containing the acetonitrile of 4 volume % was used as a developing solution using C 18-5 (250x4.6phimm). Moreover, the absorbance of 220nm detected acrylamide, methacrylamide, and a croton amide, and the absorbance of 210nm detected acrylonitrile, an acrylic acid, a methacrylonitrile, and croton nitril.

[0036] Measurement of [example 1] acrylamide concentration resistance (1)

100ml of culture media of the following presentation was prepared to the 500ml Erlenmeyer flask with a baffle, and it sterilized with the autoclave for 121 degree C and 20 minutes. After adding ampicillin so that final concentration may become this culture medium in ml and 50microg /, 1 white **** inoculation of the MT-10822 share (FERM BP-5785) was carried out, and it cultivated in 37 degree C and 130rpm for 20 hours. After centrifugal separation's (for [15000]

Gx's15 minutes) having separated only the fungus body from culture medium, then re-suspending this fungus body in a 50ml physiological saline, centrifugal separation was performed again and the wet fungus body was obtained.
[0037]
Medium composition Yeast extract 5.0 g/L The poly peptone 10.0 g/L NaCl 5.0 g/L A cobalt chloride and 6 hydrate 10.0 mg/L Ferric sulfate and 7 hydrate 40.0 mg/L pH7.5 [0038] 10mg of these wet fungus bodies was suspended in the 10g tris hydrochloride water solution (pH8.0) of 50mM(s), it added so that the final concentration of acrylonitrile might become 14% of the weight to this suspension, and the reaction was performed for 5 minutes, agitating at 10 degrees C. After adding 90g of phosphoric-acid water solutions of 10mM(s) and terminating a reaction, the acrylamide concentration in reaction termination liquid was measured by HPLC analysis. Then, the generation rate (= reaction rate) of the acrylamide per a unit wet fungus body and unit-process time amount was computed.
[0039] Then, it was made to contact for 60 minutes, suspending the 50mg of these same wet fungus bodies in 30% of the weight of the acrylamide water solution (pH8.0) containing the 40g tris hydrochloride of 50mM(s), and making them agitate gently at 10 degrees C. Then, centrifugal separation (for [15000] Gx15 minutes) separated only the fungus body from suspension. Then, after re-suspending this fungus body in the 50g tris hydrochloride water solution (pH8.0) of 50mM(s), this fungus body was washed by repeating twice actuation of performing centrifugal separation again. 10mg of these washed wet fungus bodies was suspended in the 10g tris hydrochloride water solution (pH8.0) of 50mM(s), it added so that the final concentration of acrylonitrile might become 14% of the weight to this suspension, and the reaction was performed for 5 minutes, agitating at 10 degrees C. After adding 90g of phosphoric-acid water solutions of 10mM(s) and terminating a reaction, the acrylamide concentration in reaction termination liquid was measured by HPLC analysis. Then, the generation rate (= reaction rate) of the acrylamide per a unit wet fungus body and unit-process time amount was computed.
[0040] The reaction rate before making 30% of the weight of an acrylamide water solution contact was made into 100%, and the reaction rate after making it contact for 60 minutes at 10 degrees C was measured as a relative value. Consequently, the relative value was 70%. That is, the activity survival rate was 70%.
[0041] The MT-10822 share wet fungus body was prepared by the same approach as the comparison example 1 of the concentration and the reaction rate of a [example 2] nitryl compound. 10mg of these wet fungus bodies was suspended in the 10g tris hydrochloride water solution (pH8.0) of 50mM(s), it added so that acrylonitrile might become the final concentration shown in the 1st table (Table 1) at this suspension, and the reaction was performed for 5 minutes, agitating at 10 degrees C. After adding 90g of phosphoric-acid water solutions of 10mM(s) and terminating a reaction, the acrylamide concentration in reaction termination liquid was measured by HPLC analysis. The relative value of each acrylonitrile concentration case was compared as a relative reaction rate, having computed the generation rate (= reaction rate) of the acrylamide per a unit wet fungus body and unit-process time amount, and having used the reaction rate in case the acrylonitrile concentration at the time of reaction initiation is 1 % of the weight as 100% (the 1st table (Table 1) and Fig. 1 (drawing 1)).
[0042]
[Table 1]

第1表

反応開始時のアクリロニトリル濃度（重量%）	相対反応速度
1．0	100
2．0	107
4．0	118
7．0	133
10．0	139
14．0	142
21．0	144
35．0	145

[0043] Conversion to the amide compound of a [example 3] nitryl compound (1)
The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 1.5g of these wet fungus bodies was suspended in the 98.5g tris hydrochloride water solution (pH8.0) of 50mM(s), and 36g package addition of the acrylonitrile was carried out at this suspension, and it reacted, agitating at 10 degrees C. HPLC analysis analyzed reaction mixture 8 hours after reaction initiation. Consequently, only acrylamide exists in reaction mixture (concentration = 35.0 % of the weight), and acrylonitrile was not accepted. That is, the invert ratio was 100%.
[0044] Conversion to the amide compound of a [example 4] nitryl compound (2)
The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 1.5g of these wet fungus bodies was suspended in the 98.5g tris hydrochloride water solution (pH8.0) of 50mM(s), 12g package addition of the acrylonitrile was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. Furthermore, acrylonitrile was added at the rate of 12g per hour to reaction initiation and coincidence for 2 hours, and 36g in all of acrylonitrile was added. 8 hours after reaction initiation, the same HPLC analysis as an example 1 analyzed reaction mixture. Consequently, only acrylamide exists in reaction mixture (concentration = 35.0 % of the weight), and acrylonitrile was not accepted. That is, the invert ratio was 100%.
[0045] Conversion to the amide compound of the [example 1 of comparison] nitryl compound (1)
The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 1.5g of these wet fungus bodies was suspended in the 98.5g tris hydrochloride water solution (pH8.0) of 50mM(s), and acrylonitrile was added at the rate of 2g per hour to this suspension for 18 hours. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 17.4 % of the weight, and acrylonitrile concentration was 0.8 % of the weight. That is, the invert ratio was 94%. Furthermore, the HPLC analysis same 26 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 33.0 % of the weight, and acrylonitrile concentration was 1.9 % of the weight. That is, the invert ratio was 93%.
[0046] Conversion to the amide compound of the [example 2 of comparison] nitryl compound (2)
The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 1.5g of these wet fungus

bodies was suspended in the 98.5g tris hydrochloride water solution (pH8.0) of 50mM(s), and acrylonitrile was added at the rate of 1.2g per hour to this suspension for 30 hours. When the HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture, the acrylamide concentration in reaction mixture was 10.3 % of the weight, and acrylonitrile concentration was 1.0 % of the weight. That is, the invert ratio was 88%. Furthermore, the HPLC analysis same 38 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 32.6 % of the weight, and acrylonitrile concentration was 2.1 % of the weight. That is, the invert ratio was 92%.

[0047] Conversion to the amide compound of a [example 5] nitryl compound (3)

The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 3.0g of these wet fungus bodies was suspended in the 97.0g tris hydrochloride water solution (pH8.0) of 50mM(s), 8.6g package addition of the methacrylonitrile was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. 24 hours after reaction initiation, the same HPLC analysis as an example 1 analyzed reaction mixture. Consequently, only methacrylamide exists in reaction mixture (concentration = 10.0 % of the weight), and the methacrylonitrile was not accepted. That is, the invert ratio was 100%.

[0048] Conversion to the amide compound of a [example 6] nitryl compound (4)

The MT-10822 share wet fungus body was prepared by the same approach as an example 1. 1.5g of these wet fungus bodies was suspended in the 98.5g tris hydrochloride water solution (pH8.0) of 50mM(s), 18.7g package addition of the croton nitril (however, mixture of a cis object and a trans object) was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. After dissolving completely the crystal of a croton amide which deposited 24 hours after reaction initiation at 30 degrees C, the same HPLC analysis as an example 1 analyzed reaction mixture. Consequently, in reaction mixture, only the croton amide (however, mixture of a cis object and a trans object) exists (concentration = 20.0 % of the weight), and croton nitril was not accepted. That is, the invert ratio was 100%.

[0049] Conversion to the amide compound of a [example 7] nitryl compound (5)

100ml of culture media of the following presentation was prepared to the 500ml Erlenmeyer flask with a baffle, and it sterilized with the autoclave for 121 degree C and 20 minutes. 1 white **** inoculation of the Pseudonocardia thermostat filler (Pseudonocardia thermophila) (JCM3095) given in JP,08-56684,A was carried out to this culture medium, and it cultivated in 50 degree C and 130rpm for 72 hours. After centrifugal separation's (for [15000] Gx's15 minutes) having separated only the fungus body from culture medium, then re-suspending this fungus body in a 50ml physiological saline, centrifugal separation was performed again and the wet fungus body was obtained.

[0050]

Medium composition Yeast extract 5.0 g/L Poly peptone 10.0 g/L trans-croton amide 2.0 g/L A cobalt chloride and 6 hydrate 10.0 mg/L Ferric sulfate and 7 hydrate 40.0 mg/L pH7.0 [0051] 10.0g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), 36g package addition of the acrylonitrile was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, only acrylamide exists in reaction mixture (concentration = 35.0 % of the weight), and acrylonitrile was not accepted. That is, the invert ratio was 100%.

[0052] Measurement of [example 8] acrylamide concentration resistance (2)

100ml of culture media of the following presentation was prepared to the 500ml Erlenmeyer flask with a baffle, and it sterilized with the autoclave for 121 degree C and 20 minutes. 1 white **** inoculation of J-1 share (as FERM BP-1478, it ****s to the aforementioned deposition engine based on Budapest Treaty about international acknowledgement of deposition of the microorganism on patent procedure, and sold in lots by claim to everybody) of Rhodococcus RODOKUROUSU given in JP,06-55148,B was carried out to this culture medium, and it cultivated in 30 degree C and 130rpm for 72 hours. After centrifugal separation's (15000Gx 15 minutes') having separated only the fungus body from culture medium, then re-suspending this fungus body in a 50ml physiological saline, centrifugal separation was performed again and the wet fungus body was obtained.

[0053]

Medium composition Glucose 10.0 g/L Phosphoric-acid 2 hydrogen 1 potassium 0.5 g/L Phosphoric-acid 1 hydrogen 2 potassium 0.5 g/L Magnesium sulfate and 7 hydrate 0.5 g/L A yeast extract 1.0 g/L peptone 7.5 g/L Urea 7.5 g/L A cobalt chloride and 6 hydrate 10.0 mg/L pH7.2 [0054] 10mg of these wet fungus bodies was suspended in the 10g tris hydrochloride water solution (pH8.0) of 50mM(s), it added so that the final concentration of acrylonitrile might become

14% of the weight to this suspension, and the reaction was performed for 5 minutes, agitating at 10 degrees C. After adding 90g of phosphoric-acid water solutions of 10mM(s) and terminating a reaction, the acrylamide concentration in reaction termination liquid was measured by HPLC analysis. Then, the generation rate (= reaction rate) of the acrylamide per a unit wet fungus body and unit-process time amount was computed.

[0055] Then, it was made to contact for 60 minutes, suspending the 50mg of these same wet fungus bodies in 30% of the weight of the acrylamide water solution (pH8.0) containing the 40g tris hydrochloride of 50mM(s), and making them agitate gently at 10 degrees C. Then, centrifugal separation (for [15000] Gx15 minutes) separated only the fungus body from suspension. Then, after re-suspending this fungus body in the 50g tris hydrochloride water solution (pH8.0) of 50mM(s), this fungus body was washed by repeating twice actuation of performing centrifugal separation again. 10mg of these washed wet fungus bodies was suspended in the 10g tris hydrochloride water solution (pH8.0) of 50mM(s), it added so that the final concentration of acrylonitrile might become 14% of the weight to this suspension, and the reaction was performed for 5 minutes, agitating at 10 degrees C. After adding 90g of phosphoric-acid water solutions of 10mM(s) and terminating a reaction, the acrylamide concentration in reaction termination liquid was measured by HPLC analysis. Then, the generation rate (= reaction rate) of the acrylamide per a unit wet fungus body and unit-process time amount was computed.

[0056] The reaction rate before making 30% of the weight of an acrylamide water solution contact was made into 100%, and the reaction rate after making it contact for 60 minutes at 10 degrees C was measured as a relative value. Consequently, the relative value was 95%. That is, the activity survival rate was 95%.

[0057] Conversion to the amide compound of a [example 9] nitryl compound (6)

By the same approach as an example 8, the wet fungus body of J-1 share of Rhodococcus RODOKUROUSU was prepared. 0.15g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), 60g package addition of the acrylonitrile was carried out at this suspension, and the reaction was started, agitating at 20 degrees C. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, only acrylamide exists in reaction mixture (concentration = 50.0 % of the weight), and acrylonitrile and an acrylic acid were not accepted. That is, the invert ratio was 100%.

[0058] Conversion to the amide compound of a [example 10] nitryl compound (7)

By the same approach as an example 8, the wet fungus body of J-1 share of Rhodococcus RODOKUROUSU was prepared. 0.15g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), 60g package addition of the acrylonitrile was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 47.7 % of the weight, and acrylonitrile concentration was 1.9 % of the weight. That is, the invert ratio was 95%. Furthermore, the HPLC analysis same 12 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture is 50.0 % of the weight, acrylic-acid concentration is 0.05 % of the weight, and acrylonitrile was not accepted. That is, the invert ratio was 100%.

[0059] Conversion to the amide compound of a [example 11] nitryl compound (8)

By the same approach as an example 8, the wet fungus body of J-1 share of Rhodococcus RODOKUROUSU was prepared. 0.15g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), 20g package addition of the acrylonitrile was carried out at this suspension, and the reaction was started, agitating at 10 degrees C. Furthermore, acrylonitrile was added at the rate of 20g per hour to reaction initiation and coincidence for 2 hours, and 60g in all of acrylonitrile was added. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 46.7 % of the weight, and acrylonitrile concentration was 2.6 % of the weight. That is, the invert ratio was 93%. Furthermore, the HPLC analysis same 12 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture is 50.0 % of the weight, acrylic-acid concentration is 0.05 % of the weight, and acrylonitrile was not accepted. That is, the invert ratio was 100%.

[0060] Conversion to the amide compound of the [example 3 of comparison] nitryl compound (3)

By the same approach as an example 8, the wet fungus body of J-1 share of Rhodococcus RODOKUROUSU was prepared, 0.15g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), and acrylonitrile was added at the rate of 3g per hour to this suspension for 20 hours. When the HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture, the acrylamide concentration

in reaction mixture was 23.6 % of the weight, and acrylonitrile concentration was 1.7 % of the weight. That is, the invert ratio was 91%. Furthermore, the HPLC analysis same 32 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 46.1 % of the weight, acrylic-acid concentration was 0.2 % of the weight, and acrylonitrile concentration was 2.9 % of the weight. That is, the invert ratio was 92%.

[0061] Conversion to the amide compound of the [example 4 of comparison] nitryl compound (4)

By the same approach as an example 8, the wet fungus body of J-1 share of Rhodococcus RODOKUROUSU was prepared, 0.15g of these wet fungus bodies was suspended in the 100g tris hydrochloride water solution (pH8.0) of 50mM(s), and acrylonitrile was added at the rate of 2g per hour to this suspension for 30 hours. The HPLC analysis same 8 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 16.4 % of the weight, and acrylonitrile concentration was 1.5 % of the weight. That is, the invert ratio was 89%. Furthermore, the HPLC analysis same 42 hours after reaction initiation as an example 1 analyzed reaction mixture. Consequently, the acrylamide concentration in reaction mixture was 43.5 % of the weight, acrylic-acid concentration was 0.3 % of the weight, and acrylonitrile concentration was 4.8 % of the weight. That is, the invert ratio was 87%.

[0062]

[Effect of the Invention] According to the approach of this invention, it becomes it is possible to obtain the reaction mixture of high nitril concentration in the little amount of enzymes within a short period of time more compared with the former, and possible to manufacture efficiently the amide compound which corresponds from a nitryl compound. And according to the approach of this invention, sub** of the carboxylic-acid compound corresponding to a nitryl compound is also controlled. Therefore, the approach of this invention is very useful for production of the industrial amide compound using the microorganism which produces nitril hydratase.

[0063]

[Layout Table]

[0064] array number: -- die-length [of one array]: -- mold [of 21 arrays]: -- amino acid topology: -- information: besides the description of a straight chain-like array -- partial array array Val Cys Xaa Leu Cys Ser Cys Tyr Pro Trp Pro Xaa Leu Gly Leu Pro of a nitril hydratase alpha subunit 5 10 15Pro Xaa Trp Xaa Lys 20 21 [0065] array number: -- die-length [of two arrays]: -- mold [of 20 arrays]: -- amino acid topology: -- information: besides the description of a straight chain-like array -- partial array array Val Cys Xaa Leu Cys Ser Cys Xaa Trp Pro Xaa Leu Gly Leu Pro Pro of a nitril hydratase alpha subunit 5 10 15Xaa Trp Tyr Lys 20

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

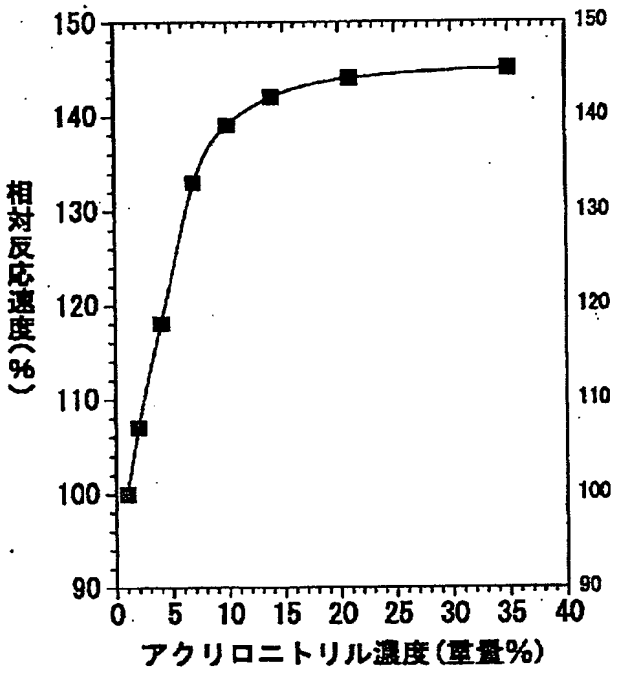
DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1] It is drawing which measured the reaction rate to the acrylonitrile concentration at the time of reaction initiation. An axis of abscissa expresses the acrylonitrile concentration at the time of reaction initiation (% of the weight), and the axis of ordinate expresses the relative reaction rate (%).

[Translation done.]

Drawing selection drawing 1

第１図



[Translation done.]

* NOTICES *

CORRECTION OR AMENDMENT

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[Filing Date] September 9, Heisei 15 (2003. 9.9)
[Procedure amendment 1]
[Document to be Amended] Specification
[Item(s) to be Amended] Claim 3
[Method of Amendment] Modification
[The contents of amendment]
[Claim 3]
The fungus body or its fungus body processing object of the microorganism which produces nitril hydratase is used. It is the approach of manufacturing methacrylamide from methacrylic nitril. In 30% of the weight of an acrylamide water solution, the fungus body of this microorganism At the time of reaction initiation of the reaction which contacts a fungus body or a fungus body processing object of this microorganism which holds the activity of nitril hydratase also after processing for 60 minutes at 10 degrees C to methacrylic nitril in an aquosity medium, and makes methacrylamide generate Or the manufacture approach of the methacrylamide characterized by adding methacrylic nitril to reaction mixture so that the methacrylic nitril concentration in the middle of a reaction may turn into more than the saturated concentration of the methacrylic nitril in the inside of an aquosity medium.

[Translation done.]